IDENTIFICATION/CONFIRMATION OF MISCELLANEOUS VOLATILES IN AQUEOUS AND BIOLOGICAL SPECIMENS BY HEADSPACE GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY MASS SPECTROMETRY

26.1 POLICY

This test method may be used to confirm the presence of miscellaneous volatiles in aqueous and biological samples. Reporting of results following the application of this method will be contingent upon a thorough review and acceptance of quality control data and the qualification of individual results under the criteria for acceptance.

Any adjustments or deviations from the procedures below must be approved by the State Toxicologist, a Manager, or a Supervisor, and appropriately documented within the batch paperwork.

26.2 PURPOSE

The purpose of this document is to describe the identification/confirmation of miscellaneous volatiles in aqueous and biological samples by headspace gas chromatography (HSGC) using an alcohol analysis capillary column and a flame ionization detector followed by injection on a gas chromatograph mass spectrometer (GC-MS). This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, and criteria for acceptance.

26.3 PRINCIPLE

There is a direct relationship between the concentration of a volatile substance (e.g. difluoroethane, butane, ethyl chloride) dissolved in a liquid (e.g. blood) and the concentration of the volatile substance in the vapor above the solution (headspace) for a given temperature, based on Henry's Law.

A sample of an aqueous or biological specimen, diluted with a measured volume of internal standard (n-propanol, 1-propanol), is measured into a vial. The vial is then sealed with a septum-equipped airtight seal. After heating, a small aliquot of the headspace is transferred to the gas chromatograph for analysis.

Preliminary identification of miscellaneous volatiles is achieved by comparison of relative retention times of observed analytes to those present in a positive control of the same volatile.

For secondary identification/confirmation, samples are injected into a GC equipped with a mass spectrometer (MS) detector. As each compound is ionized in the source, it measures the mass-to-charge ratios of each compound and its related fragments, and can be compared to the spectral pattern of a known standard or reference library.

26.4 SPECIMENS

26.4.1 The specimen volume is 0.2 mL.

26.4.2 Specimens include whole blood, serum, plasma, urine, vitreous humor, tissue homogenate, non-homogenized tissue (e.g., lung) and aqueous samples.

26.4.3 Dilutions of specimens may be analyzed at the Forensic Scientist's discretion; however, this should be done in addition to testing the standard specimen volume, unless sample quantity dictates otherwise.

26.4.4 Analysis of larger specimen volumes must be approved and documented.

26.5 EQUIPMENT AND MATERIALS

26.5.1 EQUIPMENT

26.5.1.1 Agilent (Hewlett Packard) 7694/G1888 headspace autosampler, or equivalent

26.5.1.2 Agilent (Hewlett Packard) 6890 gas chromatograph equipped with either a J&W DBALC1 capillary column (30 m x 0.53 mm ID x 3 µm film thickness) or a J&W DBALC2 capillary column (30 m x 0.53 mm ID x 2 µm film thickness), or equivalent

26.5.1.3 Agilent GC (6890 or equivalent) equipped with Restek Rtx-BAC1 30m x 0.25mm x 1.40µm column, or equivalent.

26.5.1.4 Agilent MS (5973 or equivalent)

26.5.1.5 Computer system equipped with Agilent (Hewlett-Packard) ChemStation software

26.5.1.6 Heating block, oven, or sand bath

26.5.1.7 Microlab 500 Autopipette, Hamilton Automatic Diluter, or equivalent

26.5.2 MATERIALS

26.5.2.1 Airtight syringe (manual injection)

26.5.2.2 Cap crimper

26.5.2.3 Deionized water, laboratory grade (DI H_2O)

26.5.2.4 Headspace autosampler vials (10 mL) and crimp tops

26.5.2.5 n-Propanol ISTD

26.5.2.6 Sources of miscellaneous volatiles

Note: the source of the miscellaneous volatiles may vary due to the volatile in question and are not required to be of laboratory grade. For example, butane may be obtained from a butane lighter. Difluoroethane may be obtained from a can of Dust-Off®. Ethyl Chloride may be obtained from a can of Maximum Impact® Head Cleaning solvent spray.

26.6 STANDARDS, CALIBRATORS AND CONTROLS

26.6.1 STANDARDS

24.6.1.1 Internal standard (n-propanol) is prepared and verified according to the Procedure for the Verification of n-Propanol Internal Standard.

26.6.2 CALIBRATORS

24.6.2.1 As quantitation of miscellaneous volatiles is not performed, no calibrators are required.

26.6.3 CONTROLS

26.6.3.1 Negative Control

DI H_2O is used as the sample for the negative control.

26.6.3.2 Positive Control

A diluted sample of the miscellaneous volatile serves as the positive control for retention time verification using HSGC and for spectral match comparison using GC-MS.

26.7 SAMPLE PREPARATION

26.7.1 Prepare a sample of the miscellaneous volatile for use as a positive control.

26.7.1.1 Add a small amount of the miscellaneous volatile in question into a headspace vial and immediately seal. Allow to equilibrate (~10 seconds).

26.7.1.2 Prepare two empty headspace vials with crimped caps to perform serial dilutions.

*Note: Serial dilutions are utilized to prevent saturation of the volatile on the column and to prevent contamination.

a. Using an airtight syringe, remove a full syringe of air from the equilibrated vial, and dispense it into the first of the two empty headspace vials. Allow to equilibrate (~10 seconds).

b. Using an airtight syringe, remove a full syringe of air from the first dilution vial, and dispense it into the second of the two empty headspace vials. Allow to equilibrate (~10 seconds).

26.7.1.3 Using the auto-pipetter, aliquot 200µL of DI H_2O and 2mL of n-propanol internal standard solution into a headspace vial labeled “positive control,” cap, and crimp tightly.

26.7.1.4 Using an airtight syringe, remove a syringe full from the equilibrated second serial dilution vial, and dispense it into the positive control headspace vial.

26.7.2 Label 10 mL headspace vials for each member of the test batch (blank, negative controls, specimen samples, etc.) The batch should be set up according to the following sequence, where applicable:

1. Blank 1 (DI H_2O, no ISTD)
2. Specimen
3. Negative Control (DI H_2O, plus ISTD)
4. Positive Control
5. Blank 2 (DI H_2O, no ISTD) to eliminate carryover
5. Blank 3 (DI H_2O, no ISTD) to eliminate carryover
6. Blank 4 (DI H_2O, no ISTD) to eliminate carryover

26.7.3 Equilibrate case specimens to room temperature and mix before opening under a biohazard hood. Blood specimens are inspected to ensure the blood is mobile. If necessary, the sample may be sonicated or homogenized.

26.7.4 Aliquot 2.2 mL DI H_2O into the vials labeled as blanks and seal the vials tightly.

26.7.5 Using the auto-pipetter, aliquot 200 µL of DI H_2O and 2 mL of the internal standard solution into the headspace vial as "negative control." Cap and seal the vial tightly.

26.7.6 Using the auto-pipetter, aliquot 200µL of each specimen and 2 mL of the internal standard solution into their respectively labeled headspace vials. Cap and seal the vial tightly.

Note: Non-homogenized tissue specimens (such as lung sent by a medical examiner), may be received in sealed bags or septum jars. Prepare a vial in the same manner as a negative control, sample a full syringe of air from the septum jar, and inject into the appropriately labeled headspace vial. It may be necessary to open the bag/jar and remove a small section of the lung, adding the section to the headspace vial. Preparation of non-homogenized tissue samples will be documented in the batch paperwork.

26.7.7 Between each aliquot, rinse and wash the pipette tip appropriately (e.g. rinse pipette tip with diluted bleach and/or DI H_2O. Repeat if necessary.)

26.8 INSTRUMENTAL PARAMETERS (HSGC)

26.8.1 Load and edit a sequence on the headspace gas chromatograph. Enter the blanks, controls and specimens into the sequence table. All samples are identified as Sample in Sample Type.

26.8.2 Place each headspace vial in its respective position on the headspace autosampler and verify this placement against the sequence log.

26.8.3 Run the sequence under method BLDALCO. [Note: The method name may contain a numeric suffix to differentiate between instruments; for example BLDALCO1 for headspace instrument 1. A copy of the acquisition method for each headspace instrument is available at the instrument.]

26.9 INSTRUMENTAL PARAMETERS (GC-MS)

26.9.1 Sealed headspace vials shall be heated to approximately 70°C in a heating block, sand bath, or oven prior to injection on to GC-MS.

26.9.2 All samples are manually injected into the GCMS and acquired using the method VOL.

26.10 DATA ANALYSIS

26.10.1 Analysis of the batch data is conducted using the instrumental data analysis software in ChemStation.

26.10.2 Printed reports for each vial in the batch are generated for review, along with a copy of the sequence table applied to the batch. Mass spectral matches from the GCMS shall accompany each report.

26.10.3 Technical review of the batch is conducted according to the criteria listed below.

26.11 CRITERIA FOR BATCH ACCEPTANCE

If the analysis of the batch meets the criteria listed below, the results for the specimens are accepted.

26.11.1 Blank

26.11.1.1 The blank shall be devoid of any significant peaks[1,2].

26.11.2 Controls

26.11.2.1 The negative control(s) shall be devoid of any significant peaks other than n-propanol[2]. Identification is based on acceptable retention time matching and an integrated, symmetrical peak. All negative controls must meet these criteria for the batch to be accepted.

26.11.2.2 The positive control should contain two peaks – n-propanol and the miscellaneous volatile in question[2].

26.12 CRITERIA FOR CASE SAMPLE ACCEPTANCE

If the criteria for batch acceptance have been satisfied, the results of individual case samples are deemed suitable for reporting if the following criteria are demonstrated:

26.12.1 Any chromatographic peak for the miscellaneous volatile shall appear symmetrical.

26.12.2 The retention time for the miscellaneous volatile and n-propanol are ±2% of those in the positive control. These are inclusive ranges.

26.12.3 A mass spectral match of the detected compound is compared to that in an approved mass spectral library or to a mass spectrum from the positive control. When using a library match, spectrum agreement should be 75 or greater wherever possible, taking into consideration the appearance and abundance of ions specific to that compound (an extracted ion match may be necessary). A reference spectrum for the compound found in a published article, research paper or other reference material may be acceptable if the electronic library match is not feasible, provided the source is documented.

26.13 REPORTING

26.13.1 Miscellaneous volatiles are reported qualitatively.

26.14 DOCUMENTATION AND REVIEW

26.14.1 Analysts will batch their chromatograms, sequence tables, and spectral matches together and submit the batch for both technical and administrative review. The reviewer will verify that the batch contains all chromatograms, sequence tables and spectral matches, and all dates are correctly documented. The reviewer will also verify that the batch meets the criteria for batch acceptance in 26.11 above.

[1] Peaks appearing in the blank, calibrators and negative controls that are fully resolved from the miscellaneous volatile or internal standard are considered extraneous and not significant.
[2] On the GCMS, each spectrum will show a large air peak at the beginning of each run. This is not considered a significant peak.

26.14.2 The reviewer will sign and date the batch, indicating that the batch file is complete and the above procedures have been reviewed.

26.14.3 Upon completion of the technical and administrative review, the batch is returned to the analyst.

26.14.4 If a single case sample is run for miscellaneous volatiles, all documentation is kept with the sample case file. If more than one case sample is run for miscellaneous volatiles, the blanks, positive, and negative controls will be kept in the case file for the first case analyzed.

APPENDIX A
INSTRUMENTAL PARAMETERS

GAS CHROMATOGRAPH

Split/Splitless Inlet	
Mode	Split
Inlet Liner	4 mm splitless w/glass wool plug
Split Ratio	30:1
Temperature	250°C
Gas Type	Helium
Gas Saver	On
Gas Saver Flow	15.0 mL/min
Gas Saver Time	2.00 min

Oven/Column	
Carrier Gas Mode	Constant Flow
Carrier Gas Flow	1.0 mL/min
Initial Temperature	50 °C
Run Time	10.0 min
Equilibration time	0.5 min

MASS SPECTROMETER

Solvent Delay	0.25 min	MS Quad Temperature	150 °C
EM Offset	Set in tune	MS Source Temperature	230 °C
Acquisition Mode	Scan		

LIST OF CHANGES

Revision Date	Description	Page Number
11/01/13	Method approved by Washington State Toxicologist. See DRA dated 10/29/13. Method released for use on 11/01/13.	All
10/4/16	Added “Printed Copies are Uncontrolled” to the footer.	All